REMARKS

Claims 1 and 4-14 are pending in the present application. Claims 1 and 5-13 are under consideration. Claims 4 and 14 have been withdrawn from consideration. In the present amendment, Applicants amend claims 1, 5, 6, and 10 to recite "wherein the DNA methylation pattern . . . comprises information on the methylation state of a plurality of gene regions." This language is supported in the specification, for example, at page 5, last paragraph (discussing "obtaining information on methylation"); at page 18, first paragraph under the heading "(2) RLGS technique" (discussing analysis of "[t]he state of DNA methylation"); and at page 9 (discussing identification of "cells or tissues with a combination of a plurality of spots"). Claims 1, 5, 6, and 10 also recite ". . . after obtaining the DNA methylation patterns, comparing the DNA methylation patterns thus obtained." This language is supported in the specification, for example, at page 6, first full paragraph (discussing "producing such spot patterns . . . and then comparing those spot patterns").

Thus, the amendments do not add new matter and do not raise new issues. The amendments also place the application in better condition for allowance or appeal. Thus, Applicants respectfully request entry of the amendments.

Applicants also respectfully request that the Examiner initial the Form PTO-1449, which was submitted in a Supplemental Information Disclosure Statement dated November 26, 2003, to indicate that the listed documents have been considered.

I. Rejection of Claims 1, 5, 7, and 11 Under 35 U.S.C. § 102(b)

The Examiner rejected claims 1, 5, 7, and 11 under 35 U.S.C. 102(b) as allegedly anticipated by U.S. Patent Number 5,871,917, issued February 16, 1999, to Duffy. Because Duffy fails to teach each and every element of the claims, this rejection is respectfully traversed.

The Examiner states that "Duffy determines the differences between patterns of methylation of two tested cells and therefore results in determination of the pattern of methylation of each of the tested cells." Final Office Action at page 4. The Examiner appears to argue that by merely isolating genomic regions that show differential methylation, the DNA methylation patterns of two tested cells are thus determined. However, independent claims 1 and 5 recite obtaining a DNA methylation pattern comprising "information on the methylation state of a plurality of gene regions." (See, e.g., Figure 1, which indicates whether eight different gene regions are methylated or unmethylated in three different cell types.) Duffy does not look at a plurality of gene regions to determine whether such regions are methylated or unmethylated. This information, however, is what makes up a "DNA methylation pattern," an element that is now expressly claimed. Instead, Duffy merely isolates differentially methylated genomic fragments by a subtraction method without ever obtaining DNA methylation patterns. That is, Duffy does not provide information on the methylation state of gene regions that are *not* differentially methylated, in combination with information on the methylation state of gene regions that *are* differentially methylated. Therefore, Duffy does not obtain a DNA methylation pattern that "comprises information on the **methylation state** of a plurality of gene regions."

Claims 1 and 5 further recite "comparing the DNA methylation patterns thus obtained" to identify a cell, tissue, or nucleus, or to assess the differentiation state of a cell, tissue, or nucleus. The specification provides exemplary embodiments of these aspects of the invention. For example, Figure 1 and page 4 of the specification describe the identification of cells A, B, and C by comparing methylation patterns comprising eight gene regions, five of which are differentially methylated depending on cell type. In another embodiment, Figure 5 and the accompanying text at page 9, last paragraph, describe the identification of a cell as a differentiated embryonic stem cell by comparing the cell's spot pattern with that of an undifferentiated embryonic stem cell. A spot at "position 79" is present only in undifferentiated embryonic stem cells, whereas a spot at "position 80" is present in both differentiated and undifferentiated embryonic stem cells. However, a spot at either position is not present in placenta, kidney, sperm, brain, or trophoblast stem cells. Duffy does not compare DNA methylation patterns in this manner in order to identify a cell or to assess the differentiation state of a cell.

The Examiner also states that "[t]he claims are interpreted to read on the simultaneous comparison and detection of methylation patterns of Duffy." Final Action at page 4. Claims 1 and 5 recite comparing the DNA methylation patterns "*after* obtaining the DNA methylation patterns . . . ." Thus, the obtaining of the DNA methylation patterns and the comparing of the DNA methylation patterns do not occur simultaneously. As discussed above, Duffy does not compare DNA methylation patterns, and even if *arguendo* Duffy could be interpreted as comparing DNA methylation patterns, Duffy does not compare DNA methylation patterns *after* obtaining such patterns. Therefore, Duffy does not provide this element either.

Additionally, Duffy only discusses differential methylation in cancerous cells relative to normal cells of the same cell type. See col. 11, lines 23-36 (stating that "[n]ormal cell types are cells of the same tissue-type as the 'test' cell type . . . . For example, if breast cancer cells are selected as the test cell type, the normal cell type would be breast cells that are not diseased or cancerous."). Claim 1 recites a method of identifying a cell, tissue, or nucleus by comparing its DNA methylation pattern with the DNA methylation pattern of one or more known types of cell, tissue, or nucleus. Claim 1 does not encompass identifying a cell, tissue, or nucleus as cancerous by comparing its DNA methylation pattern with that of a normal cell, tissue, or nucleus of the same type. Furthermore, the concept of identifying a cell type based on differential methylation is novel, in view of the fact that CpG islands associated with gene regions have long been believed to be generally unmethylated, and hence, would not be expected to provide information correlating differential methylation with cell type. (See enclosed article by Antequera, "CpG Islands and Methylation," *in* Nature Encyclopedia of the Human Genome (Macmillan 2003) at page 956, abstract and col. 2, and page 957, col. 2, first full paragraph.)

In view of the foregoing, Applicants respectfully request withdrawal of the rejection of claims 1, 5, 7, and 11 under 35 U.S.C. § 102(b).

II. Rejection of Claims 1, 5-7, 9-11, and 13 under 35 U.S.C. § 102(b)

The Examiner rejected claims 1, 5-7, 9-11, and 13 under 35 U.S.C. § 102(b) as allegedly anticipated by Zhu et al., Proc. Nat'l Acad. Sci. USA 96:8058-8063 (1999). Specifically, the Examiner alleges that

> Zhu et al. performed a restriction landmark genomic scanning analysis of human T cells. . . . Zhu et al. shows analysis of 1,068 fragments for methylation

> differences . . . . Zhu et al. correlate methylation differences detected by their method with types or differentiation states of the T cell lineages examined, for example in Table 3.

Final Action at pages 3-4. Because Zhu fails to teach each and every element of the claims, this rejection is respectfully traversed.

Applicants respectfully submit that the Examiner is mistaken in stating that "Zhu et al. correlate methylation differences detected by their method with types or differentiation states of the T cell lineages examined, for example in Table 3." Table 3 merely shows the selection of a single differentially methylated fragment, "fragment 0442," and correlates the spot intensity of this fragment with the level of gene expression from that fragment. At no point does Zhu "correlate methylation differences . . . with types or differentiation states of the T cell lineages . . . ." Zhu merely shows that differential DNA methylation occurs among clonal cells derived from a single T-cell. In fact, Zhu was unable to demonstrate any phenotypic differences among differentially methylated clonal cells. See page 8060, paragraph bridging columns 1 and 2. Furthermore, Zhu stated that "the mechanism causing clonal heterogeneity . . . is uncertain." Therefore, Zhu does not correlate differential methylation with cell type or differentiation state. Because Zhu does not make this correlation, Zhu can not show "a method of identifying a cell" or "a method of assessing the differentiation state of a cell" by comparing the DNA methylation pattern of the cell with that of a "known" cell, as recited in claims 1 and 5.

In view of the foregoing, Applicants submit that Zhu does not anticipate claims 1 and 5, nor does it anticipate claims 6-7, 9-11, and 13, which ultimately depend from claim 1 or 5. Withdrawal of the rejection of these claims under 35 U.S.C. § 102(b) is respectfully requested.

III. Rejection of Claims 1, 5, 7, 8, 11, and 12 Under 35 U.S.C. § 102(b)

The Examiner rejected claims 1, 5, 7, 8, 11, and 12 under 35 U.S.C. § 102(b) as allegedly anticipated by Hertz et al. J. Biol. Chem. 274:24232-24240 (1999). Specifically, the Examiner stated that

> Hertz et al. shows in the abstract the assay of methylation patterns of genes in several different mouse embryonic stem cell lines. The differences of methylation patterns in inserted genes are detailed in figures 4-9.

Final Action at 4.

Hertz investigates to what extent foreign (viral) DNA becomes methylated when it is introduced into the genome of embryonic stem cells. Hertz does not show "a method of identifying a cell" or "a method of assessing the differentiation state of a cell" by comparing the DNA methylation pattern of the cell with that of a "known" cell. Hertz is drawn to completely different subject matter. Therefore, Hertz does not anticipate claims 1 and 5, nor does it anticipate claims 7, 8, 11, and 12, which ultimately depend from claim 1 or 5. Withdrawal of the rejection of these claims under 35 U.S.C. § 102(b) is respectfully requested.

CONCLUSION

In view of the foregoing amendments and remarks, Applicants respectfully request reconsideration and reexamination of this application and the timely allowance of the pending claims. If the Examiner does not consider the application to be allowable, the undersigned requests that, prior to taking action, the Examiner call her at (650) 849-6778 to set up an interview.

Please grant any extensions of time required to enter this response and charge any additional required fees to Deposit Account 06-0916.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW,
GARRETT & DUNNER, L.L.P.

Dated: May 19, 2004

By: Danielle Pasqualone
Danielle M. Pasqualone
Reg. No. 43,847

Nature Encyclopedia of the Human Genome

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Ornithine carbamoyltransferase (*OTC*); MIM number: 311250. OMIM: http://www.ncbi.nlm.nih.gov/htbin-post/Omim/dispmim?311250

CpG Islands and Methylation

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Intermediate article

Article contents

Most CpG dinucleotides in the human genome are methylated at position 5 of cytosine. Methylated sequences are associated with transcriptional repression and an inactive chromatin conformation. In contrast, CpG islands are nonmethylated, G + C-rich regions of approximately 1 kb in length that represent approximately 1% of the genome. In the great majority of cases, they are located at the 5′ end of more than half of all human genes.

Properties and Distribution of 5-Methylcytosine in Animals

Cytosine methylated at position 5 of its pyrimidine ring is found in many eukaryotic genomes. In animals, the amount and localization of 5-methylcytosine (5-mC) varies widely. It ranges from undetectable levels in the nematode *Caenorhabditis elegans* to mosaic methylation affecting only some deoxyribonucleic acid (DNA) regions, such as in *Drosophila* and echinoderms. Among animals, vertebrates have the highest level of genomic methylation, 5-mC spreading along almost the entire genome. Because methylated cytosines are always followed by a guanine, methylation is often referred to in terms of methylated or nonmethylated CpG dinucleotides. Methylation is symmetrically organized on the DNA double helix, which means that a methylated CpG is matched by another methylated CpG in the complementary strand (Figure 1). In all, 10–20% of all CpGs are spared from methylation in mammals, which allows the existence of a potentially huge number of different methylation patterns. The information contained in these patterns can be changed without altering the nucleotide sequence and is inheritable across cell divisions by a maintenance mechanism that methylates CpGs in the daughter strand during DNA replication only when they are complementary to CpGs already methylated in the parental strand. For the above reasons, DNA methylation represents the paradigmatic and best-characterized epigenetic mechanism.

Organization and Distribution of CpG Islands in the Mammalian Genome

The methylated and nonmethylated CpGs are not randomly interspersed in the genome, but instead are segregated in two very different compartments. Most nonmethylated CpGs are clustered in the CpG islands, while methylated CpGs are scattered along the remaining 98% of the genome. CpG islands are regions that remain devoid of methylation throughout development in all tissues of the organism. In mice and humans they accounts approximately for 1% of the whole genome and, paradoxically, display a very high density of potentially methylatable CpG dinucleotides. In humans, they are approximately 1 kilobase (kb) long and have an average G + C content of 65%, which determines a frequency of approximately one CpG per 10 bp. The G + C content of the rest of the genome is 40%, and the average observed frequency of CpG dinucleotides (one CpG per 100 bp approximately) is only 20% of that expected. This is because spontaneous deamination of 5-mC generates thymine, which, during evolution, has caused the replacement of a significant number of methylated CpGs for TpGs (or CpAs in the complementary strand). The 10-fold difference in CpG density between nonisland DNA and the CpG islands readily allows their identification

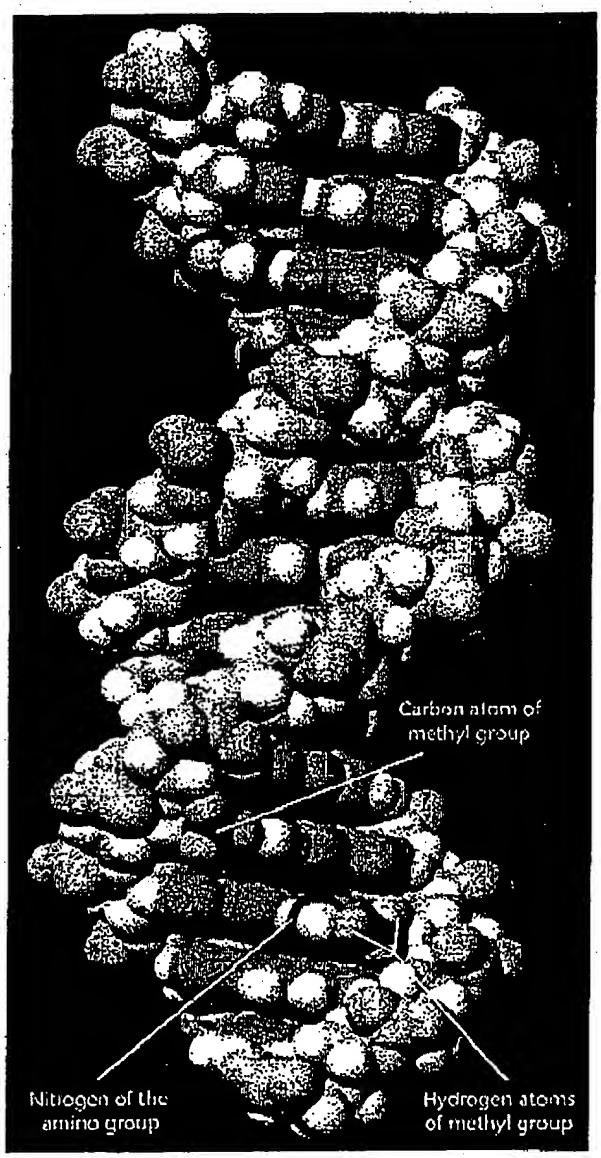

Figure 1 Three-dimensional structure of a double-stranded DNA fragment containing four methylated CpG dinucleotides. The methyl groups of the complementary methylated CpGs protrude into the major groove of the DNA molecule.

by plotting the distribution of CpGs across specific genomic regions (Figure 2). (*See* Chromosomes 21 and 22: Gene Density; CpG Dinucleotides and Human Disorders; DNA Methylation and Mutation; GC-rich Isochores: Origin; Nucleotide Substitution: Rate.)

The chromatin organization of CpG islands in the cell nucleus also has different properties relative to the bulk of the genome. Most notably, nucleosomes are positioned along the CpG island; histones H3 and H4 are highly acetylated, and the level of histone H1 is very low. Often, there are sites of hypersensitivity to deoxyribonuclease (DNase), corresponding to nucleosome-free gaps, close to the transcription initiation point. These properties, together with the absence of proteins that bind methylated CpGs (see below), confer upon the CpG islands the properties typical of what is operationally defined as 'open' or 'active' chromatin (Figure 3). (*See* Chromatin Structure and Domains.)

An essential feature of CpG islands is their association with the 5′ end of almost 60% of all human genes. This includes all housekeeping genes and approximately half of the genes with a tissue-restricted expression pattern. A few examples of CpG islands within the body of a gene or at its 3′ end have been reported. In most cases, CpG islands remain nonmethylated regardless of the expression of the associated genes. Exceptions to this general situation are the CpG islands found in the inactive X chromosome and those associated with genes subjected to genomic imprinting. In both cases, methylation of either the maternal or paternal allele is essential for mammalian development. (*See* Promoters; X-chromosome Inactivation.)

Origin and Maintenance of Methylation Patterns during Development

In all cells of female mammals, one of the two X chromosomes is inactive to compensate for the genetic-dosage differences relative to males, who only have the X chromosome inherited from their mothers. Except for a few genes that escape inactivation, the vast majority of CpG islands in the inactive X chromosome are fully methylated and adopt an inactive chromatin conformation, resulting in a transcriptional shutdown of the associated genes. Among animals, genetic imprinting is a phenomenon unique to mammals and imprinted genes are defined as those whose expression is determined by their paternal or maternal origin. In the mouse, approximately 45 imprinted genes have been identified, of which some, but not all, are also imprinted in humans. Many of them are associated with CpG islands and in most cases their allele-specific expression correlates with differential methylation. (*See* Genomic Imprinting at the Transcriptional Level; Imprinting: Evolution.)

The allele-specific methylation of CpG islands is established very early on during development. In mammals, sperm and egg genomes have a global